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TITLE: Regulation and Impact of Cytoplasmic ARID1A in Ovarian Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
ARID1A is broadly accepted to be a tumor suppressor in an increasing number of cancers, including ovarian. Silencing ARID1A in ovarian surface epithelium stimulates growth and restoration of activity in ARID1A null ovarian and endometrial cancer cells reduces proliferation, with the latter having been verified in a xenograft murine model. Immunohistochemistry studies in OCCC showed that loss of nuclear ARID1A was associated with shorter progression-free survival (PFS) and similar overall survival (OS) or with advanced stage, higher grade, suboptimal resection and nodal metastasis, though reports have been inconsistent relative to clinicopathologic associations. These prior investigations focused on nuclear localized ARID1A as a logical consequence of its tumor suppressor role in chromatin remodeling and transcriptional regulation. We recently used a well annotated ovarian cancer tissue microarray spanning histologic subtypes to evaluate the association between ARID1A and OS (N=259). Categorization of ovarian cancers by both nuclear and cytoplasmic ARID1A staining revealed a statistically significant difference in OS between the four groups with median survival times spanning more than 5 yr ($p<0.001$). In terms of the extremes, women with loss of nuclear ARID1A and prevalent cytoplasmic ARID1A had the worse survival overall with a median survival time of 7 mo. In contrast, ovarian cancer patients with loss of nuclear ARID1A without prevalent cytoplasmic ARID1A had the best OS with a median survival time of 74 mo.This Pilot Award application is focused on determining the mechanism underlying cytoplasmic quarantine of ARID1A and whether the oncogenic effect of its cytoplasmic localization is due to a simple "inactivation" of a tumor suppressor, or if ARID1A possesses a hitherto unknown oncogenic activity when localized to the cytoplasm, as is the case for other tumor suppressors, such as p21 and p27.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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Table of Contents

Page

1. **INTRODUCTION:** Ovarian cancer results in more deaths than any other gynecologic cancer in the United States. There has been little progress in improving ovary cancer therapies. Currently physicians treat most ovarian cancer patients with similar therapies regardless of the specific type of ovarian cancer. This "one size fits all" patient management occurs despite the fact that ovarian cancer is really several related but distinct diseases. Understanding these differences is vital to individualizing treatment and developing more effective, less toxic therapies. Serous ovarian cancer is the most common type of ovarian cancer and the focus of most research. The second and third most common types, namely ovarian clear cell carcinoma (OCCC) and endometrioid ovarian carcinoma (EOC), are much less studied. Whereas most patients with serous ovarian cancer may initially respond to current chemotherapies, those with EOC and OCCC are much less likely to do so. It is critical that we focus attention on these common sub-types of ovarian cancer to truly treat this disease in a personalized manner. This application is a multidisciplinary effort directly responsive to the CDMRP OCRP Pilot Award mechanism programmatic directive to "Understand the...***pathogenesis/progression*** of all types of ovarian cancer, ***including rare subtypes***" and has been formulated to result in discoveries regarding the ARID1A tumor suppressor that will make a direct and positive impact on these clinically aggressive, therapeutically refractive, and under-studied ovarian cancer sub-types. Previous reports have focused on nuclear localization of ARID1A. Within this compartment, ARID1A is known to affect nucleosomes, chromatin remodeling, gene expression and DNA repair. Our preliminary data provides clinical evidence suggesting an oncogenic role for ARID1A in ovarian cancer where it was discovered that cytoplasmically localized ARID1A is an independent prognostic factor for worse survival in this cohort of 259 ovarian cancer patients. The impact of this finding is broad, since we find that cytoplasmic localization of ARID1A retained its prognostic value in the subgroups of serous carcinoma only, women younger than 70 years old, early and advanced stage disease. In ovarian cancer patients whose tumors have loss of nuclear ARID1A with prevalent cytoplasmic ARID1A had a very grave prognosis with only a 7-month median survival time from diagnosis. Cases with loss of nuclear ARID1A in combination with negative or weak cytoplasmic localization of ARID1A had the most favorable outcome with a median survival time of 74 months. This observation was completely unexpected, but highly statistically valid. This clinically relevant preliminary data, along with our preliminary data demonstrating the ability to modulate the intracellular localization of ARID1A through selective mutagenesis of a novel bipartite nuclear localization sequence that we additionally identified, demonstrates that our group is uniquely positioned to further validate our hypothesis that ARID1A is a nuclear tumor suppressor and a cytoplasmic oncoprotein. Our work will define the mechanism by which ARID1A is mis-localized to the cytoplasm from our combined deep digital sequencing and proteomic analyses of patient tumor samples that harbor cytoplasmic ARID1A, characterize which aspects of carcinogenesis that cytoplasmic ARID1A drives, and identify the network(s) perturbed/dysregulated when ARID1A is quarantined in the cytoplasm.

2. **KEYWORDS:** ARID1A - AT rich interactive domain-containing protein 1A; tumor suppressor; ovarian cancer; SWI/SNF – switch/sucrose non-fermentable complex; ovarian clear cell carcinoma; endometrioid ovarian carcinoma

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Major Task 1: Regulatory Approval

Major Task 2: Next generation exome sequencing
Major Task 3: Global quantitative proteomic analyses
Major Task 4: Develop stable cell lines harboring ARID1A nuclear localization sequence (NLS) mutants under control of a Tet-inducible promoter
Major Task 5: In vitro studies with ARID1A NLS mutant variants.
Major Task 6: Immunoprecipitate ARID1A and analyze by LC-MS/MS

What was accomplished under these goals?

The major activities for this reporting period included pursuit of regulatory approval.

What opportunities for training and professional development has the project provided? Nothing to report.

How were the results disseminated to communities of interest? Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals? I plan to transfer this award from the Henry Jackson Foundation (HJF) to my new employer, the Inova Health System.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project? *Nothing to report*

What was the impact on other disciplines? Nothing to report.

What was the impact on technology transfer? *Nothing to report.*

What was the impact on society beyond science and technology? *Nothing to report.*

5. **CHANGES/PROBLEMS:** It has taken significantly longer than anticipated to receive regulatory approval from HRPO. A complete regulatory package was submitted to HRPO on November 9th, 2015, with frequent follow-up communications from November 2015 through February 2016. As of March 22nd 2016, the HRPO recommendation for approval is with the Approval Authority (AA) assigned to this study for final review.

Changes in approach and reasons for change. N/A

Actual or anticipated problems or delays and actions or plans to resolve them. None.

Changes that had a significant impact on expenditures. *None*

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. None

Significant changes in use or care of human subjects: None

Significant changes in use or care of vertebrate animals: None

Significant changes in use of biohazards and/or select agents: None

6. **PRODUCTS:**

Publications, conference papers, and presentations*: None*

Journal publications. *None*

Books or other non-periodical, one-time publications. *None*

Other publications, conference papers, and presentations: *None*

Website(s) or other Internet site(s)*: None*

Technologies or techniques*: None*

Inventions, patent applications, and/or licenses*: None*

Other Products*: None*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Thomas P. Conrads*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Conrads has worked to submit for regulatory approval.*
Funding Support:	This award

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Dr Conrads has transferred from the Henry Jackson Foundation to the Inova Health System. Efforts moving forward will include transfer of this award from HJF to the Inova Health System.

What other organizations were involved as partners? Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *None*